

REMARKS

The present invention features cardiac cell-specific enhancer elements derived from Csx/Nkx2.5 regulatory regions. These enhancer elements are useful, for example, for (i) regulating gene expression in cardiac cells, (ii) inducing stem cells to differentiate as cardiomyocytes, and (iii) identifying factors that induce the differentiation of stem cells as cardiomyocytes.

Examination of claims 1-17 is reported in the present Office action. Claims 13-17 are withdrawn from further consideration pursuant to 37 C.F.R. 1.142(b) as being drawn to nonelected inventions. Claims 1-12 are rejected under 35 U.S.C. § 102(b) in view of McDonald et al. (Genbank Accession Number Z64004.1, 1995; hereafter "McDonald") and Lee et al. (Genbank Accession Number AAQ79353, 1995; hereafter "Lee"), and under 35 U.S.C. § 112, first and second paragraphs. Each of these rejections is addressed below.

Amendment to the Specification

The paragraph on page 14 (line 11 to 12) of the specification has presently been amended as indicated above to replace "*mCsx/Nkx2.5*" with "*h1Csx/Nkx2.5*". No new matter has been added by the present amendment and support for the amended paragraph is found in figure 5A.

Rejections under 35 U.S.C. § 112, first paragraph

Claims 1-12 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor at the time of the application had possession of the claimed invention.

Given that the claims encompass polynucleotides having 45% to 100% sequence identity to 40 to 70 base pairs of a fragment of contiguous wild type nucleotides of SEQ ID NO: 1-6, the Examiner states that the common sequence which the nucleic acids must share to function as a cardiac-specific enhancer is unknown. This rejection has been met by the present amendments to claims 1, 9, and 11, which now recite a polynucleotide having at least 90% sequence identity to the full length sequence of both SEQ ID NO: 1 and SEQ ID NO: 2, or SEQ ID NO: 4, or alternatively 80% sequence identity to the full length sequence of SEQ ID NO: 6.

The Examiner further states that the specification fails to teach the common structural feature, which the nucleic acids must share to function as a cardiac-specific enhancer. The Examiner asserts that while the specification teaches that both the A1 and A2 domains are required for cardiac enhancer activity, it does not teach which nucleotides or common structural feature within A1 and A2 are necessary for a polynucleotide to function as a cardiac transcription enhancer. In response to the Examiner's arguments and as is discussed above, claim 1 has been amended to recite an enhancer element

sharing at least 90% sequence identity to the full length sequence of both SEQ ID NO: 1 and SEQ ID NO: 2. Given that SEQ ID NO: 1 contains the human A1 region and that SEQ ID NO: 2 contains the human A2 region, the amended claim now requires for both regions to be simultaneously present, in accordance with the teachings of the specification. Furthermore, claim 11 have been amended to recite a polynucleotide having at least 90% sequence identity to SEQ NO: 4, which includes both the human A1 and the A2 region of the cardiac enhancer. Thus, in accordance with the teachings of the present invention, claim 11 also requires the presence of a nucleotide sequence having at least 90% sequence identity to the sequence of both the A1 and A2 regions. This rejection may now be withdrawn.

The Examiner further rejects claims 1-12 for lack of enablement. The Examiner argues that the claims are broad and that the teaching of the specification is limited. In this regard, the Examiner states that the specification fails to disclose the core functional elements or motifs that are required for the nucleic acid to function as a cardiac enhancer element.

As is discussed above, the amended claims now teach a cardiac enhancer element, characterized by a nucleotide sequence having at least 90% sequence identity to both SEQ ID NO.: 1 and 2, or alternatively SEQ ID NO.: 4, and consequently containing sequence identity to both the required A1 and A2 regions. According to applicants' teachings, one skilled in the art would be able to readily test whether a polynucleotide sharing such

characteristics would result in transcriptional repressor or enhancer activity, without undue experimentation, using standard techniques in molecular biology.

For example, the specification provides extensive, enabling details concerning testing the ability of a polynucleotide sequence for its ability to function as an enhancer element. Pages 16-27 of the specification teach the identification of the enhancer element within the regulatory region of *CsX/Nkx 2.5* through the generation of transgenic animals.

Similarly, one skilled in the art could readily use such techniques to test the enhancer activity of a candidate polynucleotide sequence having at least 90% sequence identity to the sequence of both SEQ ID NO.: 1 and 2, or alternatively SEQ ID NO.: 4. Accordingly, page 18 and Example 2 of the specification on page 28-30 teach that in order

“to narrow down the cardiac enhancer, various regions of the upstream flanking sequence from the 20 kb clone were fused to the hsp68 promoter-linked *lacZ* reporter, hsp68-*lacZ* (Kothary et al., *Development* 105: 707-714 1989) (Figs. 3A-3C, 6). The expression patterns of these constructs were tested using transgenic mice to define the enhancer region of the cardiac specificity.”

Similarly, repressor activity could also be tested using the same methods as described above. Page 19 of the specification teaches the detection of a repressor element:

CsxlacZ-4 contains 6 Kb of 5' flanking sequence. Twenty-two injected embryos were analyzed, and ten found to carry the CsxlacZ-4 transgene. Interestingly, none of the CsxlacZ-4 transgenic embryos showed any β gal staining in the heart nor in any extracardiac tissues. This finding indicates that a strong negative regulatory element exist between 6 kb and 4 kb 5' to

exon 1c.

Based on the teachings and the sequences disclosed in the present invention, one skilled in the art could readily clone a polynucleotide sequence sharing at least 90% sequence identity to either SEQ ID NO: 1 and 2, or SEQ ID NO: 4, into a vector upstream of a known promoter and reporter gene such as the Lac Z gene as taught in the present invention, or in the alternative, any gene whose expression can be readily measured and quantified such as the luciferase gene. Following the insertion of the candidate transcriptional enhancer or repressor element into the expression vector, measurement of gene expression of the reporter gene would determine whether or not the polynucleotide sequence functions as a transcriptional regulatory element. If gene expression were increased above control levels (empty vector), it would follow that the sequence would have cardiac enhancer activity whereas the reverse result would be indicative of a repressor element. One skilled in the art reading the present specification would readily understand how to perform such studies without undue experimentation.

Furthermore, the Examiner asserts that although the specification teaches that the sequence of SEQ ID NO: 6 would function as a negative regulator, claim 9 on the other hand recites an **enhancer** element having 45% sequence identity to 50 contiguous nucleotides to SEQ ID NO: 6. With respect to this rejection, claim 9 has now been amended to cite a transcriptional **repressor** element having at least 80% sequence identity to the sequence of SEQ ID NO: 6.

The Examiner further asserts that claim 8 is not enabling as it would be unpredictable whether a nucleic acid comprising a random combination of any of three elements claimed would function as a cardiac enhancer. However, the Examiner fails to provide support for this rejection. Applicants note that Page 5 of the specification teaches that “the nucleic acid molecule, when operably linked to a promoter, increases activity of the promoter by at least two-fold in a cardiac cell-specific manner.” Because Applicants have provided teachings to enable the claimed invention, the burden is on the Examiner to provide evidence of the contrary. The M.P.E.P. clearly states in the Guidelines for the Examination of Patent Applications under 35 U.S.C. § 112, first paragraph, “Enablement” requirement:

A specification disclosure which contains a teaching of the manner and process of making and using an invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as being in compliance with the enablement requirement of 35 U.S.C. § 112, first paragraph, unless there is a reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support. Assuming that sufficient reason for such doubt exists, a rejection for failure to teach how to make and/or use will be proper on that basis. *In re Marzocchi*, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971). As stated by the court, “it is incumbent upon the Patent Office, whenever a rejection on this basis is made, to explain *why* it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement. Otherwise, there would be no need for the applicant to go to the trouble and expense of supporting his presumptively accurate disclosure.” 439 F.2d at 224, 169 USPQ at 370.

Thus, claim 8 is enabled by the specification. In sum, in view of the present amendments and remarks, applicants respectfully assert that claims 1-12 are fully enabled by the specification and request that the rejection under § 112, first paragraph for lack of enablement be withdrawn.

Rejections under 35 U.S.C. § 112, second paragraph

Claims 1-12 are also rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. In this respect, the Examiner points out that the term “substantially purified” renders the claims indefinite because the degree of purity of the nucleic acid is unknown, and thus, it is unclear how many and what genes the nucleic acid needs to be free from. Applicants respectfully traverse this rejection.

Applicants respectfully submit that the term “substantially purified” is clearly defined in the present specification of page 12 as

“a nucleic acid that is free of the genes, which, in the naturally-occurring genome of the organism from which the nucleic acid of the invention is derived flank the nucleic acid. The term therefore includes, for example, a recombinant nucleic acid which is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic nucleic acid of a prokaryote or eukaryotic cell; or which exists as a separate molecule (e.g., cDNA, or a genomic or cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other sequences.”

According to this definition, one skilled in the art would be able to understand the full

scope of this term. Applicants thus respectfully request that this rejection be withdrawn.

In addition, the Examiner states that the word “derive” renders claims 7 and 10 indefinite because the nature and the number of the derivative processes encompassed by the claims are unknown. Claims 7 and 10 have now been cancelled, rendering this rejection moot.

Rejection under 35 U.S.C. § 102(b)

Claim 11 is rejected under 35 U.S.C. § 102(b) as being anticipated by McDonald (1995) and Lee (1995). Claim 11 was previously drawn to a nucleic acid comprising 50 contiguous nucleotides that is at least 90% identical to 50 contiguous nucleotides of SEQ ID NO: 4 or 5. Claim 11 has now been amended to recite a polynucleotide sequence having at least 90% sequence identity to the full-length sequence of SEQ ID NO: 4. Therefore, claim 11 is not anticipated by either McDonald or Lee, since neither of the sequences of Lee or McDonald are at least 90% identical to the sequence of SEQ ID NO: 4 and thus fail to meet the required limitations of amended claim 11. Accordingly, applicants respectfully request that this rejection be withdrawn.

Conclusion

In summary, applicants submit that the claims are now in condition for allowance, and such action is respectfully requested. A marked-up version indicating the

amendments made to the specification and claims is enclosed. Enclosed is a petition to extend the period for replying for one month, to and including December 16, 2002. If there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: December 16, 2002.

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Figure 5A shows the nucleotide sequence of [*m*] *h*Csx/*Nkx2.5* homology domains A1 (SEQ ID NO.: 1) and A2 (SEQ ID NO.: 2).

Version of Claims Showing Changes Made

1. A substantially purified nucleic acid molecule comprising an enhancer element having [:

(a) 100%] at least 90% sequence identity [to 40 contiguous nucleotides of the nucleic acid molecule shown in] to the sequence of SEQ ID NO.: 1 and at least 90% sequence identity to the sequence of SEQ ID NO.:2. [or SEQ ID NO.: 3; or

(b) at least 91% identity to 50 contiguous nucleotides of the nucleic acid molecule shown in SEQ ID NO.: 2;

(c) at least 97% identity to 60 contiguous nucleotides of the nucleic acid molecule shown in SEQ ID NO.: 1 or SEQ ID NO.: 3; or

(d) at least 95% identity to 70 contiguous nucleotides of the nucleic acid molecule shown in SEQ ID NO.: 1 or SEQ ID NO.: 3.]

8. A substantially purified non-naturally occurring nucleic acid molecule having cardiac enhancer activity and comprising at least three transcription factor binding sites selected from Mef2, dHAND, GATA, TGF- β , CarG, E-box, and Csx/Nkx2.5 binding sites.

9. A substantially purified nucleic acid molecule comprising [an] a cardiac-specific repressor [enhancer] element having at least 80% sequence identity to the sequence of SEQ ID NO: 6.[:

(a) 100% identity to 50 contiguous nucleotides of the nucleic acid molecule shown in SEQ ID NO.: 6;

(b) at least 97% identity to 60 contiguous nucleotides of the nucleic acid molecule shown in SEQ ID NO.: 6;

(c) at least 93% identity to 70 contiguous nucleotides of the nucleic acid molecule shown in SEQ ID NO.: 6; or

(d) at least 90% identity to 100 contiguous nucleotides of the nucleic acid molecule shown in SEQ ID NO.: 6.]

11. A substantially purified nucleic acid molecule comprising [50 contiguous nucleotides that have a] an enhancer element having at least 90% sequence identity to the sequence of SEQ ID NO.: 4. [sequence that is that at least 90% identical to 50 contiguous nucleotides of the nucleic acid molecule of SEQ ID NO.: 4 or SEQ ID NO.: 5.]

12. [A DNA vector comprising the nucleic acid molecule of claim 1] An expression vector comprising a gene linked to a regulatory sequence wherein said sequence is a cardiac enhancer element comprising of at least 90% sequence identity to the sequence of SEQ ID NO.: 1 and at least 90% sequence identity to the sequence of SEQ ID NO.: 2, or at least 90% sequence identity to the sequence of SEQ ID NO.: 3.

Claims Pending After Entry of Amendment

1. A substantially purified nucleic acid molecule comprising an enhancer element having at least 90 % sequence identity to the sequence of SEQ ID NO: 1 and at least 90% sequence identity to the sequence of SEQ ID NO: 2.
2. The nucleic acid molecule of claim 1, wherein said element is naturally occurring.
3. The nucleic acid molecule of claim 1, wherein said element is non-naturally occurring.
4. The nucleic acid molecule of claim 1, wherein said nucleic acid molecule comprises a binding site selected from the group consisting of Mef2, dHAND, GATA, TGF- β , CarG, E-box, and Csx/Nkx2.5 binding sites.
5. The nucleic acid molecule of claim 4, wherein said nucleic acid molecule further comprises an Sp-1 binding site.
6. The nucleic acid molecule of claim 1, wherein said nucleic acid molecule, when operably linked to a promoter, increases activity of said promoter by at least two-fold in a cardiac cell-specific manner.
8. A substantially purified non-naturally occurring nucleic acid molecule having cardiac enhancer activity and comprising at least three transcription factor binding sites selected from Mef2, dHAND, GATA, TGF- β , CarG, E-box, and Csx/Nkx2.5 binding

sites.

9. A substantially purified nucleic acid molecule comprising a cardiac-specific repressor element having at least 80% sequence identity to the sequence of SEQ ID NO: 6.

11. A substantially purified nucleic acid molecule comprising a cardiac-specific enhancer element having at least 90% sequence identity to the sequence of SEQ ID NO: 4.

12. An expression vector comprising a gene linked to a regulatory sequence wherein said sequence is a cardiac enhancer element comprising at least 90% sequence identity to the sequence of SEQ ID NO.: 1 and at least 90% sequence identity to the sequence of SEQ ID NO.: 2, or at least 90% sequence identity to the sequence of SEQ ID NO.: 3.

18. The nucleic acid molecule of claim 1, wherein said enhancer element has at least 90% sequence identity to the sequence of SEQ ID NO.: 3.

19. The nucleic acid molecule of claim 1, wherein said enhancer element has at least 95 % sequence identity to the sequence of SEQ ID NO.: 1 and at least 95% sequence identity to SEQ ID NO.: 2.

20. The nucleic acid molecule of claim 1, wherein said enhancer element has at least 95 % sequence identity to the sequence of SEQ ID NO.: 3.